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(REV. 1-98)

U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

GOTE.P-039

U S APPLICATION NO (If known, see 37 CFR 1.5

09/673964

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371**

INTERNATIONAL APPLICATION NO. PCT/SE98/01468	INTERNATIONAL FILING DATE 14 August 1998	PRIORITY DATE CLAIMED 22 APRIL 1998
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TITLE OF INVENTION
METHOD FOR CHARACTERIZING SAMPLES

APPLICANT(S) FOR DO/EO/US

KUBISTA, MIKAEL

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. This is a FIRST submission of items concerning a filing under 35 U.S.C. 371
2. This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. is transmitted herewith (required only if not transmitted by the International Bureau). *Pub. PCT/WO99/57543*
 - b. has been transmitted by the International Bureau.
 - c. is not required, as the application was filed in the United States Receiving Office (RO/US).
6. A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. have been transmitted by the International Bureau.
 - c. have not been made; however, the time limit for making such amendments has NOT expired.
 - d. have not been made and will not be made.
8. A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16 below concern document(s) or information included:

11. An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. A FIRST preliminary amendment.
 - A SECOND or SUBSEQUENT preliminary amendment.
14. A substitute specification.
15. A change of power of attorney and/or address letter.
16. Other items or information:

EL556132139US

U.S. APPLICATION NO. (or Int'l. No. if Int'l.) 09/675744 INTERNATIONAL APPLICATION NO
PCT/SE98/01468

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17. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1070.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$1860.00
\$930.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$790.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4). \$720.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$98.00

ENTER APPROPRIATE BASIC FEE AMOUNT =

CALCULATIONS PTO USE ONLY

Surcharge of \$130.00 for furnishing the oath or declaration later than 20 30 months from the earliest claimed priority date (37 CFR 1.492(e)).

\$

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$
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Total claims	14 - 20 =		x \$22.00	\$
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Independent claims	1 - 3 =		x \$82.00	\$
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MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$270.00	\$
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TOTAL OF ABOVE CALCULATIONS =			\$
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Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).	+ \$
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SUBTOTAL =			\$
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Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).	\$
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TOTAL NATIONAL FEE =			\$
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Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +	\$
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TOTAL FEES ENCLOSED =			\$ 860.00
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- a. A check in the amount of \$ 860.00 to cover the above fees is enclosed.
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- c. The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 15-0610 A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:



021121

PATENT TRADEMARK OFFICE

Marina T. Larson
SIGNATURE

Marina T. Larson, Ph.D.

NAME

32,038

09/673964
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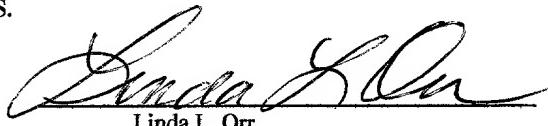
ATTORNEY DOCKET NO. GOTE.P-039
PATENT APPLICATION
October 23, 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

I hereby certify that this paper and the attachments named herein are being deposited with the United States Postal Service as Express Mail in an envelope addressed to Commissioner of Patents and Trademarks, Box PCT, Washington, D.C. 20231 on October 23, 2000. Express Mail No. EL 556132139US.

10/23/00

Date of Signature



Linda L. Orr

Applicant	:	Kubista, Mikael
Serial No.	:	TBA
International Filing Date	:	October 23, 2000
Title	:	Method for characterizing samples

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Preliminary to calculating the fees for the application filed herewith please change claims as follows:

Claim 4: Please delete the words "or 2".

Claim 7: Please delete the words "one or more of claims 1-6," and replace with the words --claim 1--.

Claim 8: Please delete the words "one or more of claims 1-7," and replace with the words --claim 1 --.

Claim 9: Please delete the words "one or more of claims 1-8," and replace with the

words --claim 1--.

Claim 10: Please delete the words "to any of claims 8 or 9" and replace with the words --to claim 8--. On line 3 of claim 10 please delete the word "thge" and substitute the word --the--.

Claim 11: Please delete the words "to any of claims 8 and 9" and replace with the words --to claim 8--.

Please add claims 12-14 as follows:

--12. A method according to claim 2, wherein one of the samples is used as a standard sample to determine the concentrations of the components in a test sample --.

--13. A method according to claim 9, for characterizing a test sample by analyzing time dependent emission/excitation spectra, where the time relates to time after excitation, time after the mixing of the components, time after a certain treatment of the components, such as chromatographic separation or the similar.--

--14. A method according to claim 9 for characterizing a test sample by analyzing two time dependencies, in combination with at least some other dependency, such as the wave length of the light, where the two time dependencies relate to time after excitation, time after the mixing of the components, time after the treatment of the components, such as chromatographic separation.--

Respectfully submitted,

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PTO/SB/08 (12-07)

Approved for use through 09/30/00. GMB 0881-0031

Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE

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**STATEMENT CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(i) & 1.27(b))—INDEPENDENT INVENTOR**

Docket Number (Optional)

Applicant, Patentee, or Identifier: MIKAEL KUBISTA

Application or Patent No.: _____

Filed or Issued: _____

Title: METHOD FOR CHARACTERIZING SAMPLES

As a below named inventor, I hereby state that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees to the Patent and Trademark Office described in:

- the specification filed herewith with title as listed above.
- the application identified above.
- the patent identified above.

I have not assigned, granted, conveyed, or licensed, and am under no obligation under contract or law to assign, grant, convey, or license, any rights in the invention to any person who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern, or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

- No such person, concern, or organization exists.
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Separate statements are required from each named person, concern, or organization having rights to the invention stating their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

MIKAEL KUBISTA
NAME OF INVENTOR

NAME OF INVENTOR

NAME OF INVENTOR

Signature of Inventor

Signature of Inventor

Signature of Inventor

02/12/15
Date

Date

Date

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3/PRTS

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METHOD FOR CHARACTERIZING SAMPLES

The present invention relates to methods for characterizing samples. These methods are i.a. used to investigate test samples from a production, patients or samples collected in any
5 other way.

Background of the invention

When a sample is to be characterized for components, the components are generally separated from each other in a first step in order to identified and quantified in a later stage.

- 10 However, it is not always possible to separate the components or it may not be motivated from a time/cost benefit reason. The samples may then be characterized spectroscopically whereby the components are identified by means of their unique spectral responses.

- If one has a collection of samples and is aware of which components they comprise, it is, as
15 a rule, trivial to determine their concentrations spectroscopically. This is due even if the spectral responses of the components overlaps each other. If, however, the components are unknown, the problem is much more complicated. The situation was analysed for the first time in detail by the mathematician Lawton and Sylvestre (Technometrics, 13, 617, (1971)), who showed that it is impossible to find an unique solution even for a 2-component system.
20 In 1990 we developed an experimental method, which partly solved this problem (Kubista, Chemometrics and Intelligent Laboratory Systems, 7, 273, (1990)). We then showed that if one carried out two spectroscopic measurements on each sample, in stead of one as previously used, and the measurements were such that the contribution of the components to these measurements had the same distribution of the intensities, but of different magnitude,
25 then both the spectral responses as well as the concentrations of the components could be determined. Mathematically, these measurements are described using the equations:

$$\mathbf{A} = \mathbf{CV} \text{ or } a_j(\lambda) = \sum_{i=1}^n c_i v_i(\lambda) \quad j = 1, 2, \dots, n$$

30 $\mathbf{B} = \mathbf{CDV} \text{ or } b_j(\lambda) = \sum_{i=1}^n c_i d_i v_i(\lambda) \quad j = 1, 2, \dots, n$

wherein **A** is a matrix comprising spectra of the first type measured on the **n** samples; **B** is a matrix comprising spectra of the second type measured on the same **n** samples; **C** is a

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- matrix comprising the concentrations of the r different components in the n samples; V is a matrix comprising the normalized spectra of the components; and D is a diagonal matrix, the r diagonal elements of which being the ratios between the responses of the components obtained in the two measurements. All spectra are digitalized in m points. We showed that
- 5 the concentrations of the components (C), their normalized spectral responses (V) and the ratio between their responses obtained in the two measurements (D) could be determined only outgoing from the information obtained from the spectra as measured (A and B). We further described how the number of components of the samples (r) could be estimated.
- 10 One restriction using this method is that the number of components are not allowed to exceed the number of samples, which from a practical point of view means that the method can not be utilized on smaller series of samples and can not be applied on the whole for analysing isolated samples.
- 15 Several spectroscopic techniques, such as fluorescence, nmr, etc., can generate 2-dimensional data described by the equation:
- $$I(\alpha, \beta) = \kappa \sum_{i=1}^r I_i(\alpha)c_i I_i(\beta)$$
- where the signal, $I(\alpha, \beta)$, is determined as a function of two variables, α and β , and are the
- 20 sum of the contribution of the components in each point, which contribution is proportional to their concentrations (c_i) and the products of their (normalized) 1-dimensional responses, $I_i(\alpha)$ and $I_i(\beta)$. Out of these responses the components can be identified. In a steady state fluorescence spectroscopy $I_i(\alpha)$ and $I_i(\beta)$ are the excitation- and emissions spectra of the components and are, as a rule, designated $I_i^{ex}(\lambda_{ex})$ and $I_i^{em}(\lambda_{em})$, wherein λ_{ex} and λ_{em} are the
- 25 excitation and emission wavelengths. The shape of an excitation spectra of a pure compound is, in general independent of the emission wavelength used at the measurement, and the corresponding is due for its emission spectrum. The fluorescence signal monitored, if necessary after a correction for the inner filter effect (Kubista et al, The Analyst, 119, 417 (1994)), is proportional to the concentration of the compound. In a sample containing more
- 30 compounds the total signal is the sum of the contribution by each component. As fluorescence is measured in an arbitrary unit, eq. 1 contains a proportionality constant (κ).

The information of the 2-dimensional spectrum $I(\alpha, \beta)$ is insufficient to unambiguously

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determine the spectral responses of the components. Different approximative ways have been suggested but these do not function sufficient satisfactorily even for a 2-component mixture (Burdick and Tu, *J. Chemometrics*, 3, 431, (1989)).

- 5 The present invention is a method for analysing isolated test samples, or a couple of test samples without using references in such a way that the components can be identified.

Description of the figures.

- Figure 1. Emission spectra monitored using different excitation wavelengths using a parallel
10 polarized light (above, left) and a perpendicularly polarized light (above right), respectively. Down to the left the calculated emission spectra of the components are shown, and down to the right the calculated excitation spectra of the components are shown.

- Figure 2. A) Excitation spectra registered using different emission wavelengths from two
15 solutions containing POPOP, dimethyl POPOP, antracene, and diphenyl antracene. B) The excitation spectra of the components as calculated.

- Figure 3. A) Emission spectra registered using different excitation wavelengths of two
solutions containing POPOP, dimethyl POPOP, antracene, and diphenyl antracene. B) The
20 excitation spectra of the components as calculated.

Brief description of the invention

- The present invention is a method for analyzing test samples in such a way that its components can be identified without the need for any reference data. The method is based upon
25 the following four steps:

1. The test sample is analyzed using a method generating a 3-dimensional response accord-

ing to : $I(\alpha, \beta, \gamma) = \sum_{i=1}^r \tilde{I}_i(\alpha) \tilde{I}_i(\beta) \tilde{I}_i(\gamma)$,

- wherein r is the number of components contributing to the signal, and $\tilde{I}_i(\alpha)$ and $\tilde{I}_i(\beta)$ and
30 $\tilde{I}_i(\gamma)$ are the arbitrarily normalized 1-dimensional responses of the components, which responses normally consist of spectral or concentration variations.

2. The number of components r as the samples contain is estimated.

3. For each component its 1-dimensional responses $I_i(\alpha)$ and $I_i(\beta)$ and $I_i(\gamma)$ are determined.

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4. Out of the responses, the components are identified.

Detailed description of the present invention

As the title indicates the present invention relates to a method for characterizing isolated test samples in a way that makes it possible to identify its components without any need for using reference samples. This is done through a strategic design of experiments which makes it possible to register a 3-dimensional response being proportional to the concentrations of the components, and the contribution from each component is the product of its specific 1-dimensional responses:

$$10 \quad I(\alpha, \beta, \gamma) = \sum_{i=1}^r c_i I_i(\alpha) I_i(\beta) I_i(\gamma)$$

Such registration can be carried using certain forms of fluorescence spectroscopy, e.g., by means of a time disintegrated monitoring of emission/excitation spectra, i.e., the signal is registered as a function of excitation wavelength, emission wavelength, and time:

$$15 \quad I(\lambda_{ex}, \lambda_{em}, t) = \sum_{i=1}^r c_i I_i(\lambda_{ex}) I_i(\lambda_{em}) I_i(t)$$

In these cases it is often suitable to gather the concentration of the components c_i and the time declinations to a time dependent concentration:

$$I(\lambda_{ex}, \lambda_{em}, t) = \sum_{i=1}^r c_i(t) I_i(\lambda_{ex}) I_i(\lambda_{em})$$

20 The time can be time after light pulse (whereby $c_i(t)$ is proportional to the fluorescence declination), time after mixing of e.g., a stop-flow experiment (whereby $c_i(t)$ is the variation of the concentration of component i with time), time after treatment, such a photo bleaching (selective destruction of certain components using light), chromatographic or other form of separation, etc. At the analysis of such data the concentration variation of the components
25 are calculated, as well as their excitation and emission spectra. It is of interest to note that intermediate components which are neither present at the beginning ($c_i(0) = 0$) or at the end ($c_i(\infty) = 0$) of the experiment can be identified from its calculated spectra.

There is a further possibility in varying the polarization of the light:

$$30 \quad I(\lambda_{ex}, \lambda_{em}, \alpha) = \sum_{i=1}^r c_i I_i(\lambda_{ex}) I_i(\lambda_{em}) I_i(\alpha)$$

or, if the phase-modulated light is utilized, the frequency of the modulation:

$$I(\lambda_{ex}, \lambda_{em}, \nu) = \sum_{i=1}^r c_i I_i(\lambda_{ex}) I_i(\lambda_{em}) I_i(\nu)$$

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etc.

There is further a possibility in varying the outer parameters which influences the concentrations of the components, such as temperature (pressure, volume, etc.):

$$5 \quad I(\lambda_{ex}, \lambda_{em}, T) = \sum_{i=1}^l c_i(T) I_i(\lambda_{ex}) Y_i(\lambda_{em})$$

or outer parameters which influence the intensity of the responses of the components, such as external magnetic fields (electrical fields, etc.):

$$10 \quad I(\lambda_{ex}, \lambda_{em}, M) = \sum_{i=1}^l c_i I_i(\lambda_{ex}) Y_i(\lambda_{em}) I_i(M).$$

- The spectroscopic technique need not be a fluorescence technique. The method can be carried out using most techniques which generates 3-dimensional responses, e.g., nuclear magnetic resonance spectrometry (NMR) mass spectrometry, etc. It can further be carried out using most techniques generating 2-dimensional responses if the responses of the components influence external parameters. Finally, the method can be used using a technique generating 1-dimensional responses, as well, but then it is necessary that two external parameters are varied simultaneously and that their influence on the responses of the components are independent so that their contribution can be factorized.

- 20 The invention requests that at least two data points are determined in each of the 3 dimensions, i.e.:

$$I_i(\alpha) \text{ wherein } \alpha_1, \alpha_2, \dots, \alpha_n \geq 2$$

$$I_i(\beta) \text{ wherein } \beta_1, \beta_2, \dots, \beta_m \geq 2$$

$$I_i(\gamma) \text{ wherein } \gamma_1, \gamma_2, \dots, \gamma_l \geq 2$$

25

To determine two data points only in all dimensions are, however, of particular meaning as the tolerance of the responses calculated then as a rule is insufficient to be able to identify the components. On the contrary it is quite excellent to have two data points only in one of the dimensions, e.g., $l=2$ (and $m \gg 2$, and $n \gg 2$). This exhibits the advantage that the numerical treatment of data is made easier as the responses of the components can be calculated

- 30 using fast algorithms such as Procrustes rotation and GRAM (Kubista, Chemometrics and Intelligent Laboratory Systems, 7, 273, (1990); Wilson, Sanches & Kowalski, J. Chemometrics, 3, 493, (1989)). In the general case when all l , n , and m are greater than 2, the

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solution method is much more complicated and thus considerably more time consuming (Liwo, et al, Computers Chem., 21, 89-91, (1997)). Furthermore, it is quite often of interest to carry out the experiment in such a way that one of m and n are considerably greater than the other. The reason hereto is that it as a rule, is sufficient for the identification of the components, to determine one of their 1-dimensional responses with a high accuracy.

The invention is not limited to determinations that generates 3-dimensional responses but even responses of a higher order can be used. In general it should be satisfying that the response is linear and that the contribution from each component shall be the product of its 10 1-dimensional responses:

$$I(\alpha, \beta, \gamma, \delta, \dots) = \sum_{i=1}^r c_i I_i(\alpha) I_i(\beta) I_i(\gamma) I_i(\delta) \dots$$

Of course, the higher the dimension is the more time consuming the numerical treatment of 15 the determined data will become. However, with regard to the very fast development within the computer area this will hardly be a practical limitation in the future.

The samples to be analysed shall contain substantially the same components, and these shall be present in different, relative concentrations. The samples are analysed in pair using a 2-dimensional method which provides a response which is proportional to the concentrations 20 of the components and the product of the 1-dimensional responses. This can be expressed as:

$$I^A(\alpha, \beta) = \sum_{i=1}^r I_i(\alpha) c_i^A I_i(\beta)$$

$$I^B(\alpha, \beta) = \sum_{i=1}^r I_i(\alpha) c_i^B I_i(\beta)$$

wherein $I^A(\alpha)$ and $I^B(\beta)$ are spectra of the two samples which in the following will be called A and B, determined as a function of the variables α and β , r is the total number of components contributing to the spectra, $I_i(\alpha)$ and $I_i(\beta)$ are the normalized 1-dimensional responses 30 of the components, and c_i^A and c_i^B are their concentrations, respectively. In a steady-state fluorescence spectroscopy $I_i(\alpha)$ are the normalized excitation spectra of the components, $I_i^{ex}(\lambda_{ex})$, and $I_i(\beta)$ are their normalized emission spectra, $I_i^{em}(\lambda_{em})$:

$$I^A(\lambda_{ex}, \lambda_{em}) = \sum_{i=1}^r I_i^{ex}(\lambda_{ex}) c_i^A I_i^{em}(\lambda_{em})$$

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$$I^B(\lambda_{ex}, \lambda_{em}) = \sum_{i=1}^r I_i^{ex}(\lambda_{ex}) c_i^B I_i^{em}(\lambda_{em})$$

The information in these spectra is treated in two steps. First the number of components, r ,
 5 is determined, and then the 1-dimensional responses of the components,
 $I_i^{ex}(\lambda_{ex})$, and $I_i^{em}(\lambda_{em})$.

When r has been determined, the spectral responses of the components.

10 The equations: $I^A(\lambda_{ex}, \lambda_{em}) = \sum_{i=1}^r I_i^{ex}(\lambda_{ex}) c_i^A I_i^{em}(\lambda_{em})$

$$I^B(\lambda_{ex}, \lambda_{em}) = \sum_{i=1}^r I_i^{ex}(\lambda_{ex}) c_i^B I_i^{em}(\lambda_{em})$$

can be written in matrix form as:

$$A = X C^A M$$

15 $B = X C^B M$

wherein A and B are matrixes comprising the spectra determined, X is a matrix comprising the normalized excitation spectra of the components, M is a matrix comprising their normalized emission spectra, and C^A and C^B are diagonal matrixes comprising the concentrations of the components. By renormalizing one of X or M , the equation system can be rewritten
 20 as:

$$A = X M$$

$$B = X D M$$

wherein D is a diagonal matrix comprising the ratios between the concentrations of the components ($D = C^B / C^A$). Using A and B , X , M and D can be calculated using known
 25 methods such as Procrustes rotation (Kubista, Chemometrics and Intelligent Laboratory Systems, 7, 273, (1990); and GRAM (Wilson, Sanches & Kowalski, J. Chemometrics, 3, 493, (1989).

As a summary, the present invention relates to a method for experimentally studying two
 30 samples spectroscopically so that the information present in the experimental spectra is sufficient to determine the number of components of the samples (r), their spectral responses of the 1st dimension, $I_i(\alpha)$, their spectral responses of the 2nd dimension, $I_i(\beta)$, and the ratios between their concentrations (c_i^A / c_i^B).

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The most apparent use of the invention is for the analysis of two samples containing common components. All components need not be common, but the majority of those contributing spectroscopically should be in common (Booksh & Kowalski, J. Chemometrics, 8, 287, (1994)). The number of components is arbitrary and can exceed 2.

5

Another use of the invention is to characterize single samples by first dividing them into two part samples containing the ingoing components in different proportions. This can be accomplished in several ways, e.g., by filtering, extracting, chromatographying, dialysing, centrifuging, precipitating, splitting the sample by means of an electrical field, etc. Alter-

10 natively, the original sample can be used as one sample, and an aliquot thereof, which is created in such a way that the components are present in other proportions, is used as the second sample. This aliquot can be obtained by selectively eliminating certain components, e.g., by means of adsorption, precipitation, freezing, distillation, selective decomposing (e.g., by light, heat, radio lysis), etc. Another possibility is to create two samples from one,

15 is to change the conditions for the determination, e.g., by changing the temperature, pressure, etc. Separation methods, such as different types of chromatography are of interest, as the components are separated in space, and one, principally arbitrary number of samples can be obtained which can be analysed in pair. Using spectroscopic techniques which generates 2-dimensional spectra in a fast way, then, furthermore, the detection can be made on-line.

20

Another use of the invention is to determine the concentrations of the components in one test sample in relation to a standard sample with a high degree of accuracy. The standard sample and the test sample are analysed as a pair, and the ratio between the concentrations of the components is obtained as the diagonal element of the **D** matrix.

25

2-dimensional spectra wherein one of the dimensions is time, are of particular interest, whereby time is related to time after a disturbance such as a relaxation time. Today, there are e.g., fluorescence instruments by means of which one can determine complete spectra as a function of time after lightening (either directly after lightening using a light pulse, or indirectly using phase modulation technique). This gives using α as time, and β as wave length, the equation system:

$$I^A(t, \lambda) = \sum_{i=1}^c I_i(t) c_i^A I_i(\lambda)$$

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$$I^B(t, \lambda) = \sum_{i=1}^r I_i(t) c_i^B I_i(\lambda)$$

from which r, $I_i(t)$, $I_i(\lambda)$ and (c_i^A/c_i^B) can be determined.

5 Example

The invention will be further illustrated in four examples.

Example 1

A sample is characterized using fluorescence spectroscopy, where excitation wave length, 10 emission wave length, and light polarization are varied (Figure 1). This gives raise to a 3-dimensional spectrum according to:

$$I(\lambda_{ex}, \lambda_{em}, \alpha) = \sum_{i=1}^r c_i I_i(\lambda_{ex}) I_i(\lambda_{em}) I_i(\alpha)$$

In the example 650 different emission wave lengths (m), 11 different excitation wave 15 lengths (n) and 2 different polarizations ($\alpha = 0^\circ$, called parallel polarization, and $\alpha = 90^\circ$, called perpendicular polarization) (l), are used. From the response determined, $I_n(\lambda_{ex}, \lambda_{em}, \alpha)$, first the number of components (r) is estimated to 2 (using a statistic test and a visual inspection of the principal components). Then the component specific responses are calculated. For this purpose one uses the fact that only two data points were registered in one 20 of the dimensions (polarization) and rewrote the 3-dimensional response to two 2-dimensional responses.

$$I(\lambda_{ex}, \lambda_{em}, \alpha = 0^\circ) = \sum_{i=1}^r c_i I_i(\lambda_{ex}) I_i(\lambda_{em}) I_i(\alpha = 0^\circ)$$

$$I(\lambda_{ex}, \lambda_{em}, \alpha = 90^\circ) = \sum_{i=1}^r c_i I_i(\lambda_{ex}) I_i(\lambda_{em}) I_i(\alpha = 90^\circ)$$

25 These can be described using the equation system:

$$I^0 = X \alpha^0 M$$

$$I^{90} = X \alpha^{90} M$$

which can be solved using Procrustes rotation (Kubista, Chemometrics and Intelligent Laboratory Systems, 7, 273, (1990)). This gave the normalized excitation intensities of the components as matrix $X(I_i(\lambda_{ex}))$, (shown down to the right in Figure 1), the normalized emission intensities of the components as matrix $M(I_i(\lambda_{em}))$ (shown down to the left in Figure 1), and the ratios between the responses of components to light of different polarization

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From the calculated component specific responses, in particular the emission spectra, the component could be identified as p-bis[2-(5-phenyloxazolyl)]-benzene (POPOP), and antracene. Finally, by comparing standard spectra of POPOP and antracene the concentrations could be estimated to some micro molar.

5

Example 2

Two solutions containing the dye compounds POPOP, dimethyl POPOP, antracene and diphenyl antracene in different proportions were prepared. On these fluorescence excitation spectra were monitored at several emission wave lengths. The number of components were 10 determined to 4 using a statistic test, and the excitation spectra of the components (Figure 1), emission intensities and the relation between their concentrations in the two samples were calculated.

Example 3

15 On the same solutions as in Example 1 the fluorescence emission spectra were monitored using a number of excitation wave lengths . The number of components was determined to 4 using a statistic test, and the emission spectra of the components (Figure 2), excitation intensities and the relation between their concentrations in the two samples were determined.

Example 4

Characterization of samples containing the dye compound thiazole orange and the polymer poly(dG) was made. The samples were analysed in pairs using 2-dimensional fluorescence spectroscopy. They contains thiazole orange and poly(dG) in the relation [thiazole orange]/[poly(dG)] of 0.05 and 0.025. Neither poly(dG) nor the dye compound is 25 fluorescentic as such but the fluorescence arises when thiazole orange binds to the polymer. The samples were analysed in two different ways. In one analysis, the fluorescence excitation spectra were monitored at different emission wave lengths. The number of fluorescent components were identified to two using statistic tests, and their excitation spectra and emission intensities were calculated.. In the second analysis, the fluorescence emission 30 spectra were monitored using a number of excitation wave lengths. Once again the number of components was identified to two, and their emission spectra and excitation intensities were calculated.

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CLAIMS

1. A method for characterizing a sample,
characterized in that
 a) a sample, or pair of samples, is (are) characterized using a monitoring technique such that
 5 a multi dimensional response is generated according to

$$I(\alpha, \beta, \gamma, \delta, \dots) = \sum_{i=1}^r c_i I_i(\alpha) I_i(\beta) I_i(\gamma) I_i(\delta), \dots,$$

- b) the response monitored is broken down to an orthogonal basis e.g., using a principal component division,
 10 c) the number of components (r) in the sample is estimated,
 d) the arbitrary normalized 1-dimensional responses of the components are calculated.

2. A method according to claim 1, wherein the number of samples is two and these are analysed using a method generating a 2-dimensional response according to

$$15 \quad I(\alpha, \beta) = \sum_{i=1}^r I_i(\alpha) c_i I_i(\beta)$$

and the 1-dimensional responses of the components and the ratios between their concentrations in the two samples, (c_i^A/c_i^B), is calculated by solving the equation system

$$20 \quad I^A(\alpha, \beta) = \sum_{i=1}^r I_i(\alpha) c_i^A I_i(\beta)$$

$$I^B(\alpha, \beta) = \sum_{i=1}^r I_i(\alpha) c_i^B I_i(\beta)$$

3. A method according to claim 2, wherein the two samples are generated from one sample.
 25 4. A method according to claim 1 or 2, wherein one of the samples is used as a standard sample to determine the concentrations of the components in a test sample.

5. A method according to claim 1, wherein a single sample is analysed using a technique generating 3-dimensional response:

$$30 \quad I(\alpha, \beta, \gamma) = \sum_{i=1}^r c_i I_i(\alpha) I_i(\beta) I_i(\gamma)$$

and the arbitrary normalized 1-dimensional responses of the components, $\tilde{I}_i(\alpha)$ and $\tilde{I}_i(\beta)$ and $\tilde{I}_i(\gamma)$ are calculated.

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6. A method according to claim 1, wherein a single sample is analysed using a technique generating a 2-dimensional response simultaneously as external parameters are varied in such a way that the concentration of the components are changed in time:

$$I(\alpha, \beta, \gamma) = \sum_{i=1}^r c_i(t) I_i(\alpha) I_i(\beta)$$

5 and the arbitrary normalized 1-dimensional responses, $\tilde{I}_i(\alpha)$ and $\tilde{I}_i(\beta)$ and their changes as to concentration $c_i(t)$ is calculated.

7. A method according to one or more of claims 1-6, wherein more than two data points are
10 monitored in only two of the dimensions.

8. A method according to one or more of claims 1-7, wherein the method generating the multi dimensional response is fluorescence or nuclear magnetic resonance method.

- 15 9. A method according to one or more of claims 1-8, wherein the variations along, at least one of the dimensions, is obtained by varying one external parameter, such as time, electrical or magnetical field, temperature, modulation, or polarisation

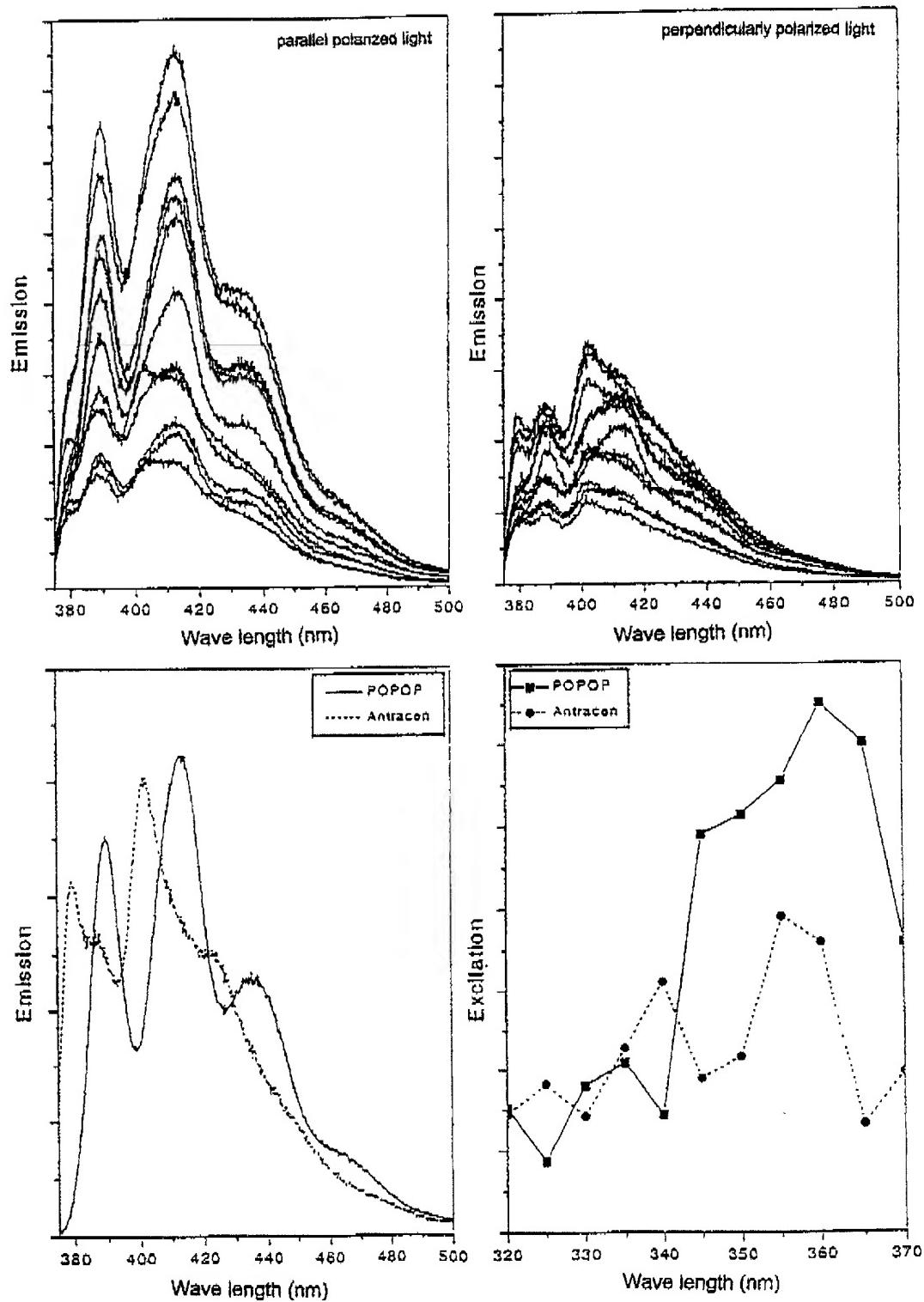
10. A method according to any of claims 8 or 9, for characterizing a test sample by analysing
20 time dependent emission/excitation spectra, where the time relates to time after excitation, time after the mixing of the components, time after a certain treatment of the components, such as chromatographic separation or the similar.

11. A method according to any of claims 8 and 9 for characterizing a test sample by analysing
25 two time dependencies, in combination with at least some other dependency, such as the wave length of the light, where the two time dependencies relates to time after excitation, time after the mixing of the components, time after the treatment of the components, such as a chromatographic separation.

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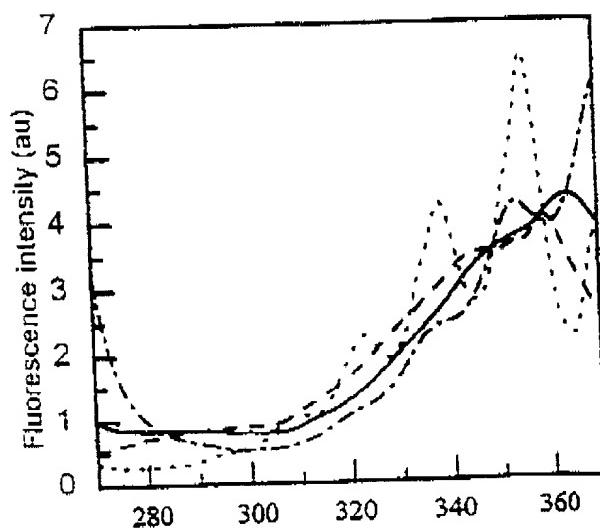
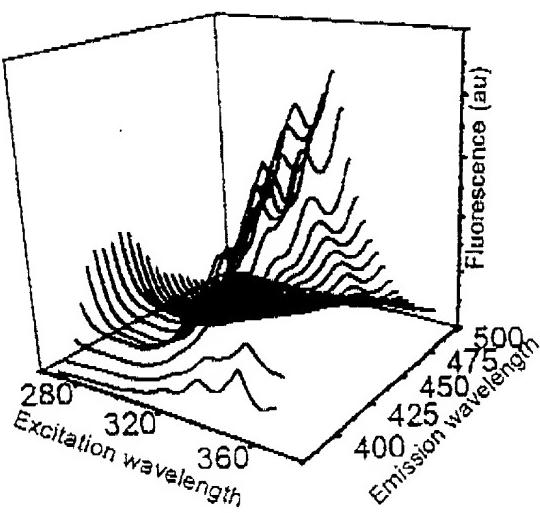
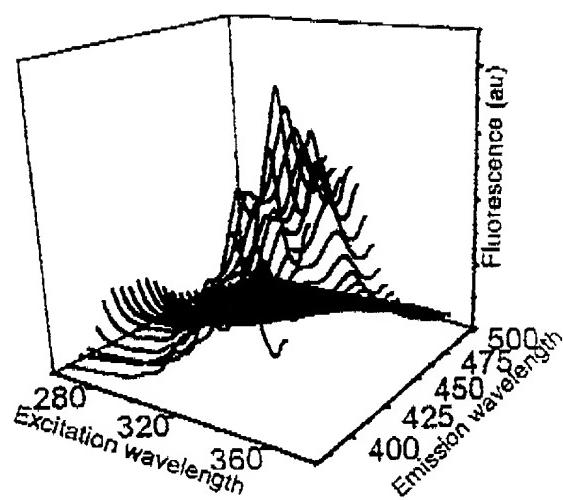
FIGUR 1

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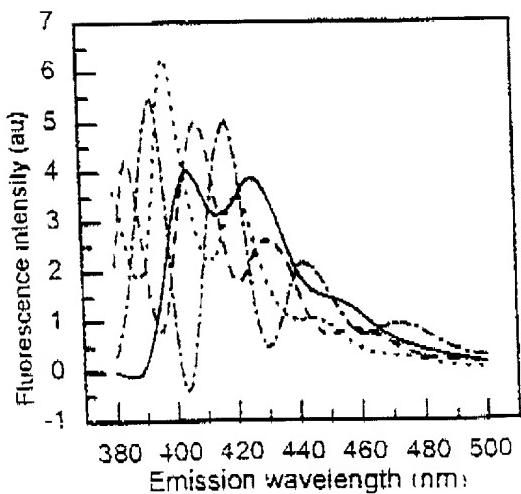
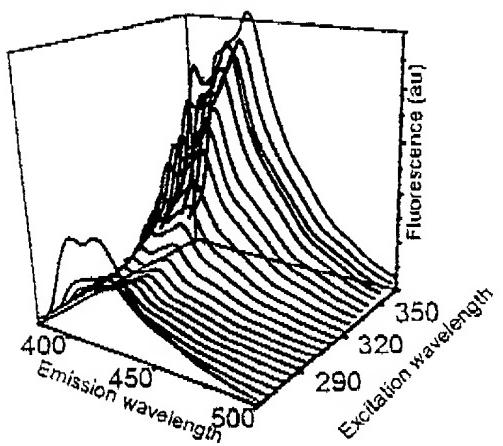
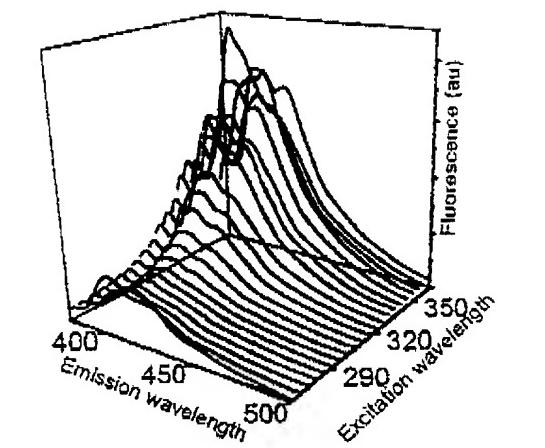
FIGUR 2

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FIGUR 3

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter, which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR CHARACTERIZING SAMPLES

the specification of which

[] is attached hereto.

[] was filed on _____ as United States Application Number or PCT International Application Number _____ and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information, which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate, or any PCT international application having a filing date before that of the application on which priority is claimed:

Prior foreign application(s)			
Country	Application Number	Date of Filing (day/month/year)	Priority Claimed
SWEDEN	9801420-2	22-04-1998	YES [X] NO []
			YES [] NO []

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

Application Number: Filing Date:
Application Number: Filing Date:

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

U.S. Parent Application Serial Number:

Parent Filing Date:

Parent Patent No.:

U.S. Parent Application Serial Number:

Parent Filing Date:

Parent Patent No.:

PCT Parent Number:

Parent Filing Date:

POWER OF ATTORNEY: I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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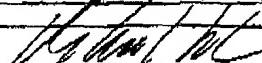
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Additional inventor's are being named on separately numbered sheets attached hereto.